Decontaminating Infectious Laboratory Waste by Autoclaving

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The temperature profile of infectious laboratory waste being autoclaved was examined relative to the type of containers used in the process. A standardized waste load (1,750 ± 4 g) placed in the container was evaluated by using a direct readout thermocouple. The sensor of the thermocouple was placed within an unused and outdated agar plate, centrally located about 5 cm from the bottom of the container. The gravity displacement autoclave tested reached 121°C within 3 min. Waste within a steel container (plus 1 liter of water) reached 108, 120, and 122°C at 12, 30, and 50 min, respectively. Without the addition of water, the corresponding temperatures were 60, 110, and 120°C, respectively. With a steel container, "autoclavable" plastic bags, and no additional water, the temperatures were 36, 71, and 105°C, respectively. When 1 liter of water was placed in the autoclavable bag, the temperatures were 98, 115, and 121°C, respectively. Waste within a polypropylene container (dimensions similar to those of the steel container) with and without the addition of 1 liter of water, reached a maximum temperature of 108°C at 50 min. With a polypropylene container, autoclavable plastic bag, and 1 liter of water, the corresponding temperature was 99°C. Without the addition of water, the temperature was 92°C. The importance of container, moisture, and material in autoclaving was demonstrated.

Infectious laboratory waste is generated in microbiology diagnostic laboratories and from facilities conducting research involving the use of pathogenic agents. Generally, such waste is decontaminated on location to minimize the health risk associated with its final collection, handling, and disposal.

Autoclaving (i.e., saturated steam under pressure) infectious waste is recommended (1, 8, 11) and widely practiced because it is considered to be the most reliable and easily controlled process for on-location decontamination. Although we agree with this recommendation, it must be noted that conditions necessary to achieve adequate decontamination are not described (1) or if described are not supported by sufficient data (3, 4, 7, 12). Loading conditions that have an adverse effect on achieving the desired thermal conditions in waste being autoclaved have been noted (6, 11). Unfortunately, avoiding such conditions is not sufficient to assure that the waste has reached a temperature of at least 115°C for 20 min. This, in the authors' opinion, is the time-temperature condition necessary for adequate decontamination of infectious waste.

This lack of information on conditions necessary for decontamination and the autoclaving process described in the "Proposed Hazardous Waste Regulation" (5) of the Environmental Protection Agency prompted this study.

MATERIALS AND METHODS

The time-temperature profile of laboratory waste being autoclaved was determined with a temperature probe (Yellow Springs Instrument Co., model 427x) attached to a direct readout recorder (Yellow Springs Instrument Co. Tele-thermometer, model 42SF).

An initial evaluation was conducted in several locations, using different autoclaves, to determine whether a temperature gradient occurred in the waste during the autoclave process and, if so, the coolest location. This was determined by attaching the sensing elements of four temperature probes at specific locations (5, 10, 15, and 20 cm) from the end of a wood dowel. Biological (Minnesota Mining and Manufacturing, At-test no. 1242) and chemical (Bio-Medical Sciences Inc., Thermalog S) indicators were also attached to the dowel at the same locations as the sensing element. Subsequently, the wood dowel was inserted centrally through the waste to the bottom of the container. The waste was then processed in gravity type displacement autoclaves that operated at an internal temperature of from 121 to 123°C.

Processing time was preset for 50 min. However, no effort was made to control other autoclaving practices. The chemical and biological indicators were removed after the waste had been processed. The visual results from the chemical indicators were recorded immediately. The biological indicators were incubated at 56°C for 48 h and then read for spore survival.

The primary evaluation was conducted to determine the effects of waste loading conditions. The time-temperature profile of the waste being processed was determined by inserting the sensing element of a

temperature probe into an outdated and unused plastic disposable petri dish (100 by 15 mm) containing agar. This dish was then centrally located 5 cm from the bottom of a container. The waste load (1,750 or 3,500 g) consisted of disposable plastic petri dishes (100 by 15 mm) containing agar. This load was placed in either a stainless steel (thickness, 0.13 cm) or a polypropylene (thickness, 0.25 cm) container with similar dimensions and approximately a 12-liter (13 quarts) capacity. A given amount (0, 100, or 1,000 ml) of water was added to the container before processing. In addition, the waste load was placed in a 0.008-cm (3 ml) polypropylene "autoclavable" waste bag (Adamco, 46 by 61 cm). A given amount (0, 100, or 1,000 ml) of water was added to the bag, which was then placed in either the steel or the polypropylene container and processed.

A gravity type displacement autoclave (AMSCO, model 57CR) was used during this evaluation. Processing time was preset for 50 min. The internal temperature of the autoclave reached 121° to 122°C within 3 minutes after initiating its cycle.

At least three trials of each loading condition were conducted. Similar waste loads were not autoclaved during the same trial.

RESULTS

Data from the initial evaluation showed that the time-temperature profile in a waste load, regardless of waste composition, was related to the height of the waste container. For containers of about 28 cm in height, the lowest temperature profile occurred 5 cm from the bottom of the container. The lowest profile in a 61-cm container was observed about 10 cm from the bottom. The temperature profiles invariably increased with an increase in height. A typical example of time-temperature gradient profiles in a waste load is presented in Fig. 1.

The results from the biological and chemical indicators showed good correlation with recorded low and high time-temperature profiles. For 36 observations in which the maximum temperature of the waste was less than 112°C, both types of indicators showed that the decontamination process was inadequate. For 29 observations in which the temperature was greater than 118°C for 30 min, both types of indicators showed that the process was adequate. However, the results of the indicators varied greatly when the temperature in the waste was between 113 and 117°C for less than 30 min. The 15 biological indicators were negative for spore survival (i.e., the process was adequate). The 15 chemical indicators showed various degrees of thermal processing; however, all showed that the process was inadequate.

The results when the waste loading conditions were controlled are presented in Fig. 2, 3, 4, and 5. The average temperatures of the waste in a steel container (plus 1 liter of water) were 108, 120, and 122°C at 12, 30, and 50 min, respective-
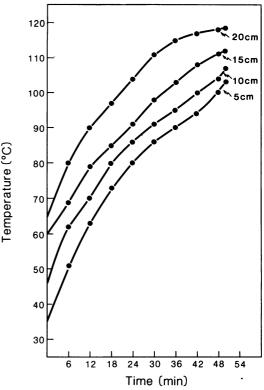

FIG. 1. Time-temperature gradient profiles in a waste load that was autoclaved at 121°C for 50 min. The thermocouples were located 5, 10, 15, and 20 cm from the bottom of the waste containers.

ly (Fig. 2). Without the addition of water, the corresponding temperatures were 60, 110, and 120°C, respectively. With a steel container, autoclavable plastic bag, and no additional water, the temperatures were 37, 71, and 105°C, respectively. When 1 liter of water was placed in the autoclavable bag, the temperatures were 98, 115, and 121°C, respectively.

Figure 3 shows the average time-temperature profiles of waste processed in polypropylene containers. Waste within a polypropylene container with and without the addition of 1 liter of water reached a maximum temperature of 108°C at 50 min. With a polypropylene container, autoclavable bag, and 1 liter of water, the corresponding temperature was 99°C. Without the addition of water, the temperature was 92°C.

Table 1 shows the average temperature and standard deviation (degrees Celsius) observed in the steel and polypropylene containers during three trials. Note that the standard deviations in the steel container with or without an autoclavable bag and one liter of water were less than 2.4 18 min into the process.
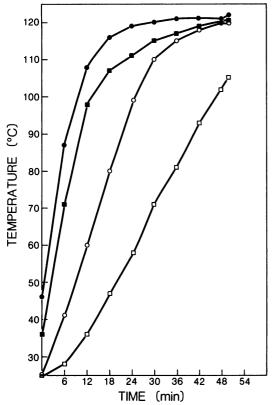
FIG. 2. The average time-temperature profiles of waste loads (1,750 g) consisting of petri dishes (100 by 15 mm) containing agar. The loads were autoclaved in a steel container with (●) or without (○) 1 liter of water and in a steel container with an autoclavable plastic waste bag with (■) or without (□) 1 liter of water. Averages are based on three separate trials conducted in a gravity type displacement autoclave that reached 121 to 122°C within 3 min after initiating the cycle.

The effect of the amount of water added to waste in an autoclavable bag that was processed in a steel container is shown in Fig. 4. The average temperatures of the waste containing 1,000, 100, and 0 ml of water were, at 24 min, 111, 90, and 57°C, respectively. After 50 min, the corresponding temperatures were 120, 113, and 106°C, respectively.

The average time-temperature profile was depressed (after 24 min of processing) by about 3 to 8°C when the amount of waste being processed was doubled (Fig. 5). Note that this is only for the addition of 1 liter of water to an autoclavable bag in a steel container.

DISCUSSION

Infectious laboratory waste can be adequately decontaminated (i.e., waste temperature of at least 115°C for 20 min) in a gravity displacement autoclave operating at 121°C. However, the processing time necessary to achieve this result is dependent on specific waste loading conditions.

Our study shows that a processing time of 50 min is adequate when waste is placed either directly into a steel container with the addition of 1 liter of water or into an autoclavable waste bag (plus 1 liter of water), which in turn is placed into a steel container. When water is not added to the waste in a steel container or an autoclavable bag in a steel container, a processing time of greater than 50 min is needed. The use of a polypropylene container will substantially increase the processing time, regardless of the addition of water. The worst case, relative to
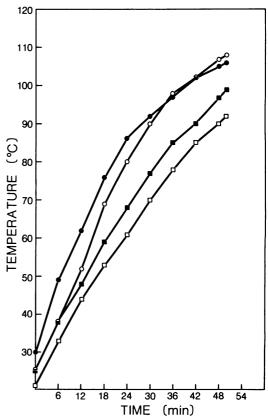
FIG. 3. The average time-temperature profiles of waste loads (1,750 g) consisting of petri dishes (100 by 15 mm) containing agar. The loads were autoclaved in a polypropylene container with (●) or without (○) 1 liter of water and in a polypropylene container with an autoclavable plastic waste bag with (■) or without (□) 1 liter of water. Based on three separate trials conducted in a gravity type displacement autoclave that reached 121 to 122°C within 3 min after initiating the cycle.
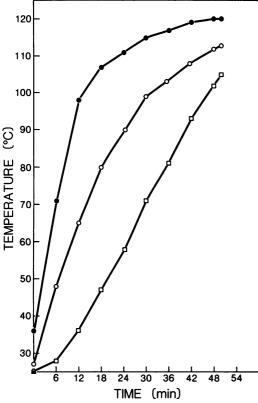
FIG. 4. The average time-temperature profiles of waste loads (1,750 g) consisting of petri dishes (100 by 15 mm) containing agar. The loads were autoclaved in a steel container with 1 liter (●), 100 ml (○) or 0 ml (□) of water. Based on three separate trials conducted in a gravity type displacement autoclave that reached 121 to 122°C within 3 min after initiating the cycle.

TABLE 1. Average temperature of waste observed in steel and plastic containers during three trials

Condition	Avg temp (°C) and SD at various times (min)									
	0	6	12	18	24	30	36	42	48	50
Steel container	25 (4.1)	41 (15.6)	60 (21.6)	80 (15.6)	99 (6.3)	110 (2.6)	115 (2.0)	118 (0.6)	120 (0.6)	120 (0.6)
Steel container + 1 liter of water	46 (12.5)	87 (11.5)	108 (4.4)	116 (2.3)	119 (1.5)	120 (1.5)	121 (0.6)	121 (0.6)	121 (0.6)	122 (0)
Steel container + autoclavable bag	24 (0.6)	28 (2.6)	36 (7.2)	47 (11.9)	58 (12.8)	71 (14.8)	81 (12.0)	93 (8.5)	102 (3.6)	105 (5.0)
Steel container + autoclavable bag + 1 liter of water	36 (9.6)	73 (9.6)	98 (3.0)	108 (0.7)	112 (1.2)	115 (1.2)	117 (0.6)	119 (0.6)	120 (1.0)	121 (0.6)
Plastic container	25 (1.5)	38 (9.5)	52 (11.3)	69 (10.0)	80 (7.0)	90 (4.7)	98 (3.5)	102 (2.0)	107 (1.0)	108 (1.0)
Plastic container + 1 liter of water	30 (6.8)	49 (17.9)	62 (20.5)	76 (15.3)	86 (9.2)	92 (7.0)	97 (3.0)	102 (1.5)	105 (0.6)	106 (1.1)
Plastic container + autoclavable bag	21 (1.5)	33 (6.6)	44 (8.3)	53 (9.5)	61 (9.8)	70 (8.9)	78 (6.8)	85 (5.8)	90 (5.1)	92 (3.7)
Plastic container + autoclavable bag + 1 liter of water	25 (3.0)	38 (13.5)	48 (14.5)	59 (15.6)	68 (14.0)	77 (12.6)	85 (8.8)	90 (6.0)	97 (5.1)	99 (4.3)

length of processing time, was that of autoclavable bags, with no water added, placed in polypropylene containers.

We believe that the results are readily explained by thermal dynamic principles. For example, the thermal conductivity of various steels is 100 to 500 times greater than that of polypropylene (2). Water in appropriate containers (steel or autoclavable bag) facilitates heat transfer in that its thermal conductivity is 20 to 30 times greater than that of air. In addition, the heated water causes increased convective heat transfer through moist vapor which permeates the waste load. It is extremely important to overcome the cool, dry air pockets in the waste, which occur in upright containers (10) or autoclavable bags when a gravity displacement type autoclave is used.

Our study also indicated that the chemical indicator was as reliable in monitoring adequate
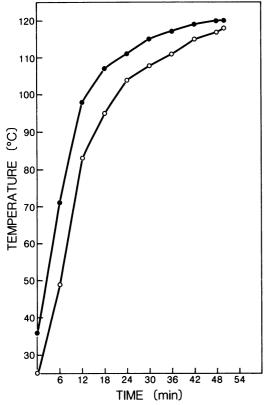
FIG. 5. The average time-temperature profile of waste loads of 1,750 g (●) or 3,500 g (○) consisting of petri dishes (100 by 15 mm) containing agar. The loads were autoclaved in a steel container with 1 liter of water. Based on three separate trials conducted in a gravity type displacement autoclave that reached 121 to 122°C within 3 min after initiating the cycle.

decontamination as the biological indicator. In fact, we prefer the chemical indicator because the result is available immediately after the autoclaving process. This is important when one considers that decontaminated waste is handled and disposed of before the availability of results from the biological indicator (i.e., 48 h). Howev-

er, one should note that not all chemical indicators on the market are reliable (9).

We suggest that each laboratory review its present practice to insure that the processing time is sufficient to decontaminate infectious waste adequately. Also, extreme caution must be used when adding water to infectious waste so that aerosols containing infectious agents are not generated. Water should be trickled gently down the side of the container rather than poured in directly.

LITERATURE CITED

1. Block, S. S., and J. C. Netherton. 1977. Infectious hospital wastes: their treatment and sanitary disposal, p. 738. In S. S. Block (ed.), Disinfection, sterilization and preservation, 2nd ed. Lea and Febiger, Philadelphia.
2. Brookes, R. 1969. Properties of materials suitable for the cultivation and handling of micro-organisms, p. 50. In J. R. Norris and D. W. Ribbons (ed.), Methods in microbiology, vol. 1. Academic Press Inc., London.
3. Centers for Disease Control. 1974. Disposal of solid wastes from hospitals. Hospital Infectious Branch and Microbiologic Control Branch, Bacterial Disease Division, Bureau of Epidemiology, Atlanta, Ga.
4. Dole, M. 1978. Warning on autoclavable bag. American Society for Microbiology News 44:283.
5. Environmental Protection Agency. 1978. Hazardous waste: proposed guidelines and regulations and proposal on identification and listing. Fed. Regr. 43:58946–59208.
6. Ernst, R. R. 1977. Sterilization by heat, p. 481–521. In S. S. Block (ed.), Disinfection, sterilization and preservation, 2nd ed. Lea and Febiger, Philadelphia.
7. Everall, P. H., and C. A Morris. 1976. Failure to sterilize in plastic bags. J. Clin. Pathol. 29:1132.
8. Howie, J. 1973. Sterilization of discarded cultures. J. Appl. Bacteriol. 36:i.
9. Lee, C. -H., T. J. Montville, and A. J. Sinskey. 1979. Comparison of the efficacy of steam sterilization indicators. Appl. Environ. Microbiol. 37:1113–1117.
10. Perkins, J. J. 1972. Principles and methods of sterilization in health science, 2nd ed. Charles C Thomas, Publisher, Springfield, Ill.
11. Richardson, J. N., and R. H. Huffaker. 1980. Biological safety in the clinical laboratory, p. 960–964. In A. Balow, W. J. Hausler and J. P. Truant (ed.), Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
12. Rubbo, S. D., and J. F. Gardner. 1965. A review of sterilization and disinfection. Lloyd-Luke Ltd., London.